Remarks/Arguments

The withdrawal of all previously outstanding rejections (under 35 USC §§ 102(b), 112, 1st paragraph and 112, 2nd paragraph) is respectfully acknowledged.

Applicant and his representative thank the Examiner for the courtesy of a telephonic interview on August 25, 2003. With respect to the rejection under § 102, Applicant pointed out that priority should be granted back to the earliest filing date, with the result that the cited reference should be cited as prior art under § 102(a). The Applicant stated his reasons why the reference should not be considered anticipatory, but indicated that a 131 amendment would be submitted to speed prosecution. Also discussed were the claim recitations that resulted in new rejections under § 112, first paragraph. Applicant explained why the disputed limitations should not be considered new matter and/or need not be present in the claims. Applicant's positions are set forth below in detail.

Claims 33-34, 36-37, 43-50, 59-65, 71-78, and 87-90 are pending in the application and stand rejected. Claims 33, 43, 59, 63, 71, and 87-89 are amended.

The Claimed Invention

The claimed invention is fully supported by instant specification and by the original specification filed as U.S. Ser. No. 07/154,206, filed Feb. 10, 1988, and issued as U.S. Patent No. 4,980,281 (the "'281 patent"). What is disclosed and claimed is a method for determining whether a chemical agent is a direct inhibitor or activator of an enzyme-of-interest (more generally, a protein-of-interest, or "POI") in a cell. The method provides a means by which one of ordinary skill in the art can identify substances that are specific inhibitors or specific activators, *i.e.*, substances that both bind to the POI and inhibit or activate the cellular function of the POI, as opposed to a wide variety of other proteins or non-protein targets in the cell. As stated in the first paragraph of the Summary of the Invention, the method involves "the generation of a cell line purposefully engineered to detect both stimulatory and inhibitory agents which are absolutely specific for any given protein . . . ." (Specification, page 3, line 34 - page 4, line 4; '281 patent, Col. 2, lines 27 - 32). The effect of such compounds on the cellular functioning of the POI is reflected in modulation of a responsive phenotypic characteristic exhibited by the cell which becomes

greater with increasing expression of the POI. (*See, e.g.*, Specification, page 12, lines 22 - 28; '281 patent, Col. 6, lines 14 - 20) The phenotypic characteristic is "responsive" in that it is a phenotypic characteristic of the cell that is modulated by activators or inhibitors of the POI and whose modulation is responsive to the level of the protein in the cell. The latter is also referred to in several places in the Specification as a "graded cellular response."

It is this choice of a defined phenotypic response, evoked by the presence and functional activity of the POI under defined conditions, that results in an assay that inherently identifies substances that bind to the POI, rather than elsewhere, in order to exert their inhibitory or activating effect on the cellular functioning of the POI.

Prior to Applicant's invention, one of ordinary skill would have expected that substances found to have an effect on such a test cell and modulate the responsive phenotypic characteristic would exert this effect by acting on one or more of many cellular components *other than* the POI. In fact, at the time, Applicant's concept of using a cellular response-based asay system to identify chemical agents that bind to the POI in order to exert their effects was considered *impossible*. Indeed, one longstanding problem in drug discovery was determining the mode of action of any substances found to have pharmaceutical activity. Prior to Applicant's invention, in order to identify substances that bind to and modulate the activity of a POI, one of ordinary skill in the art would have, for example, 1) screened for substances that bind to the POI (*e.g.*, using a cell-free purified protein binding assay or an intact cell binding assay, neither of which involve assaying the cellular functioning of the POI), and subsequently tested each substance for pharmacologic activity *in vivo*, or 2) screened substances for pharmacologic activity and subsequently attempted to identify which cellular component was the actual target of the compound.

The present method relies on the Applicant's conception and discovery that if a cell line is established in which a POI is overproduced such that a *responsive change in a phenotypic characteristic* (*i.e.*, the "*phenotypic response*") is properly defined and characterized as extensively disclosed in Applicant's teachings, then that defined phenotypic characteristic will be found to responsively change when the cell is exposed to chemical agents that bind to and inhibit or bind to and activate the POI. Therefore, this phenotypic

response of the cell can be used to identify substances that bind to the POI and modulate its cellular activity. Furthermore, Applicant also showed for the first time that by using such a test cell that stably overproduces a POI, one can further expect that substances found to modulate the previously defined responsive phenotypic characteristic of the cell will be doing so by binding to the POI and modulating its specific activity in the cell.

As shown below, the Specification establishes and emphasizes in numerous places the importance of the level of expression of the POI and its relationship to the responsive change in a phenotypic characteristic that is observable in response to activators or inhibitors of the POI.

The sensitivity of a test cell of the invention is dependent on the production of the POI. For example, the Specification (page 4, lines 13 - 16; '281 patent, Col. 2, lines 40 - 44) recites that "[t]he sensitivity of the cells is dependent on the production of the POI, a phenomenon referred to herein as a "graded cellular response" to the pharmacological agent [that activates or inhibits the POI - see Specification, page 4, lines 6 - 11; '281 patent, Col. 2, lines 34 - 39]." The test cells stably overproduce the POI (Specification, page 4, lines 32 - 35; '281 patent, Col. 2, lines 57 - 60).

Another recitation (page 10, lines 25 - 28; '281 patent, Col. 5, lines 39 - 42) explains, "[t]he host cells should exhibit a readily observable phenotypic change as a result of enhanced production of the POI. Preferably, this response should be proportional to the level of production of the POI. Finally, the cells should not spontaneously mainfest the desired phenotypic change." At page 12, lines 22 - 28 ('281 patent, Col. 6, lines 14 - 20) it is recited:

> What we are looking for is a increase in the phenotypic change exhibited by the cell which becomes greater with increasing expression of the POI. We call this a "graded cellular response," and it is by this specialized response that we distinguish inhibitors or activators of the POI from agents that act upon other cell metabolites to effect a phenotypic change.

Further, regarding exemplified test cells that stably overexpress PKC, the specification recites, "[t]he cell lines which resulted from the application of this method are highly sensitive and responsive both to agents which activate PKC as well as to those which inhibit PKC." (Specification, page 23, lines 3-6; '281 patent, Col. 10, lines 26 - 29) That

PKC expression is stable in a preferred embodiment is clearly stated (*e.g.*, "Isolation of cell lines stably overexpressing PKC," Specification, page 25, line 23 - page 26, line 18; '281 patent, Col. 11, line 45 to Col. 12, line 7). In the context of the cell-based assay method of the invention, the overproduction of the β1 isoform of PKC is sufficient to enable the test cells to remain responsive even after repeated exposures to TPA, a known activator of PKC that will down-modulate PKC activity in a cell expressing typical wild-type levels of the enzyme. For example, in cells expressing normal levels of PKC, it is known that endogenous levels of PKC are reduced upon addition of the PKC activator. Therefore, the control cells of Example 1 returned to their normal appearance by 48 hours after exposure to TPA and failed to respond to a second dose of TPA. In contrast, test cells retained their altered morphology in the presence of TPA and would responsd once again to renewed doses of TPA. (Specification, page 34, line 19 - page 36, line 5; '281 patent, Col. 16, lines 1-55).

Accordingly, the following characteristics, *inter alia*, are inherent in any embodiment of the invention:

1) The phenotypic characteristic that changes in response to activators and inhibitors of the POI is assayable at least under conditions wherein the POI is stably overproduced;

2) A responsive change in a previously defined phenotypic characteristic that is observed in the presence of a test substance results primarily from a change in the activity of the POI, rather than from a change in the amount of the POI;

3) Maintenance of the level of the POI provides the skilled investigator with the ability to define a phenotypic characteristic of the test cell that is capable of responding to substances that modulate the activity of the POI. Test cells of the invention are able to exhibit the phenotypic response following removal and subsequent retreatment of the cells with a so called "direct" activator or inhibitor of the enzyme.[1] The effects of down modulation of the POI are overcome.

[1] Throughout his patents and patent file histories, the Patentee has referred to specific inhibitors or specific activators of a selected protein of interest as "inhibitors of the POI" or "activators of the POI," respectively. Consistent with standard usage in the scientific literature, and the understanding of one of ordinary skill in the art in the context of describing the relationship between a substance and its protein, specificity requires, by definition, that a substance bind to (*i.e.*, interact with; interact directly with) the POI in order to be a specific inhibitor or a specific activator, *i.e.*, an inhibitor or and activator *of the POI*.

Priority

Priority has been granted back to August 10, 1989, the filing date of U.S. Ser. No. 08/392,073. Applicant respectfully asserts that the claimed invention is entitled to priority back to February 10, 1988, which is the filing date for U.S. Ser. No. 07/154,206, issued as U.S. Patent No. 4,980,281. The instant application claims methods for determining whether a chemical agent is a direct inhibitor or activator of an enzyme in a cell whose production by that cell evokes a responsive change in a phenotypic characteristic of the cell, other than the level of the enzyme in the cell *per se*. Throughout prosecution of the instant application, Applicant has provided parallel citations to the instant specification and the specification of the '281 patent to show support for the claims and all recited limitations.

Rejection Under 35 U.S.C. § 102

Claims 33-34, 36-37, 43-44, 46, 47, 49-50, 63-65, 71-72, 74-75, 77-78 and 88-89 stand rejected under 35 U.S.C. § 102(b) as anticipated by Riedel et al. ("Riedel").

Applicant's Prior Invention

In view of the priority date of February 10, 1988 to which Applicant believes the invention is entitled, Riedel should be considered a reference under § 102(a). Riedel appeared in the April 10, 1987 issue of Science. Consistently, the receipt date stamped on the cover page of that issue by the University of Wisconsin Biomedical Library is April 13, 1987. Further, Applicant's representative has been informed that copies of Science became available at the offices of the American Association for the Advancement of Sciences on the issue date, and that mailing to subscribers commenced on the same day. Accordingly, Riedel was publically available as a reference beginning no earlier than April 10, 1987, which is less than a year before Applicant's priority date.

Thus, solely for the purpose of speeding prosecution, Applicant asserts that the claimed invention was conceived prior to publication of Riedel and diligently reduced to practice, as established by the accompanying Declaration of Dr. Gerard M. Housey Under 37 C.F.R. § 1.131, the inventor of this subject matter. In his declaration, Dr. Housey provides the requisite evidence showing that the claimed invention was conceived prior to publication

of Riedel and diligently reduced to practice in the United States at least as early as June 13, 1987. Hence, Riedel is removed as a reference. The anticipation rejection is rendered moot and withdrawal is respectfully requested.

Riedel does not Anticipate the Claimed Invention

Aside from Applicant's declaration with respect to Riedel, Applicant wishes to respond to the Examiner's comments and point out why Riedel, like other prior art that has been previously raised in the PTO and elsewhere, neither anticipates nor suggests the claimed invention. Riedel creates a hybrid receptor consisting of the external and transmembrane domains of the human EGF receptor (EGFR) fused to the cytoplasmic tyrosine kinase domain of the avian erythroblastosis virus *erb*B oncogene product. Notably, the cytoplasmic domain of human EGFR is homologous to v-*erb*B cytoplasmic domain. The authors' rationale for their experimental approach is based on this sequence homology together with the apparent evolutionary loss of the extracellular ligand binding domain of the v-*erb*B oncogene product thought to be responsible for the latter's transforming capability (Abstract, lines 6-7, and p. 200, last paragraph).

Completely apart from an assay to determine whether a substance is a direct activator or a direct inhibitor of the hybrid receptor, Riedel starts with a substance (EGF) that *a priori* binds to the external domain of EGFR, and confirms with a direct binding assay that the hybrid receptor is still capable of binding EGF. Further assays to investigate the *in vitro* or *in vivo* functioning of the hybrid receptor are performed with full knowledge that it is still capable of binding EGF. The "integrity" of the intracellular domain is then tested *in vitro* by analyzing autophosphorylation activity in immunoprecipitates of detergent cell lysates (Legend, Fig. 1B). The cellular functioning of the hybrid receptor is then characterized in assays that suggest that EGF may be both an activator and an inhibitor of cell growth. However, none of the assays identifies a responsive change in a phenotypic characteristic of the cell that is conclusively linked to the cellular functioning of the hybrid receptor, which is central to Applicant's invention. Thus, Riedel provides no guidance or suggestion as to how any cellular function of the hybrid receptor can be used to identify other substances that bind to the hybrid receptor and inhibit its function or that bind to the hybrid receptor and activate

its function. In contrast, Applicant's claimed invention is a method for determining (identifying, screening) whether a substance binds to and inhibits a POI (direct inhibitor; inhibitor of the POI) or binds to and activates a POI (direct activator; activator of the POI) in a cell.

Riedel has not identified and characterized a phenotypic response according to Applicant's invention. This property is, by definition, a characteristic of a test cell of the invention - a characteristic first recognized by the Applicant to provide a means to identify and distinguish test substances that bind to the POI in order to modulate its activity versus other mechanisms or ways for a substance to exert a cellular effect. Riedel neither defines a phenotypic response according to Applicant's teachings nor demonstrates that such a phenotypic response can be used as a tool to identify novel inhibitors or activators of a given POI. Riedel has not demonstrated that any identified characteristics of the hybrid EGF receptor bearing Rat 1 cells, including biochemical characteristics such as autophosphorylation of the hybrid receptor (Fig. 1B), or physiologic characteristics such as mitogenic responses of the transfected cells (Fig. 2A), foci formation (Fig 2B), or anchorage-independent growth (Fig. 3) could be used to determine whether any substance other than EGF is capable of binding to the hybrid receptor and is able to act as an inhibitor or an activator of the hybrid receptor. Any conclusions they can draw from their experiments are, in their own words, related to determining whether this hybrid protein is able to retain some of its functional activity (p. 197, Abstract, last sentence; see also detailed discussion below).

The Examiner has indicated that Riedel analyzed phosphorylation of an intracellular protein substrate of the hybrid enzyme as a responsively changing phenotype of the cells. Applicant respectfully points out that what is measured by Riedel is autophosphorylation, the process whereby a protein kinase phosphorylates itself on serine, threonine or tyrosine residues, rather than phosphorylating a downstream target of said protein kinase. Furthermore, these authors have studied autophosphorylation of the hybrid receptor in an *in vitro* assay using cell lysates (see legend to Fig. 1B). The change in phosphorylation of the hybrid receptor in response to treatment with a substance in a cell-free assay (comprised of cell lysates) is not a change in a phenotypic response of the cell because it is observed *in vitro*

in an assay where the state of the hybrid receptor has already been divorced from any effect on substrates or cellular functions. (The cells are lysed prior to addition of ligand and detection of autophosphorylation.) No downstream intracellular substrate of the hybrid receptor is identified or analyzed whatsoever.

Applicant agrees with the Examiner that phosphorylation of a downstream intracellular protein substrate (*i.e.*, not the POI itself) is potentially a relevant phenotypic response of test cells of the invention, so long as such a property is identified and characterized as such according to Applicant's teachings. However, Riedel did not do this. Riedel demonstrated that EGF affects autophosphorylation of the hybrid receptor in a cell free assay, but disclosed no phenotypic response of the cell involving phosphorylation of a substrate of the hybrid receptor.

Furthermore, one of ordinary skill in the art knows that autophosphorylation by itself is not indicative of functioning of a given POI in cellular signal transduction systems. For example, it has been shown that a mutated receptor protein kinase may retain its autophosphorylation activity *without* being able to propagate an intracellular signal at all (See, e.g., White et al., 1988, Cell 54:641-9, cited in the Applicant's U.S. Pat. No. 5,266,464 at Col. 30, lines 22 - 25, Exhibit A, previously disclosed). One could not use Applicant's method to create a cell-based assay system with such a mutant receptor since the protein has lost its ability to induce a cellular response under any conditions. Even if the mutant POI retains its kinase activity and is able to autophosphorylate, if it cannot be induced to evoke a phenotypic response in the cell under a defined set of conditions then it cannot be used to create a cell-based assay system according to Applicant's method. Likewise, because the protein kinase activity of Riedel's hybrid receptor is not established as acting on a substrate or another characteristic of the cell in a defined and predictable manner, the hybrid receptor has not been established as a POI according to the invention.

Lastly, it is worth emphasizing that, in contrast to Riedel's autophosphorylation assay, Applicant's method involves treatment of an intact test cell with a test substance. Once the intact cell has been treated with a substance, there is no limitation according to Applicant's method as to how the skilled investigator may measure (assay) the change in a given

characteristic of the cell, and such measurement will frequently involve disrupting the cell in order to perform the measurement (such as measuring cAMP levels, phoshoinositide levels, etc, ion flux, reaction products produced by an enzyme, etc.). However, at the beginning of Applicant's assay, since it is a cellular response that is being followed, the intact cell must be treated with the test substance.

Turning to the other potential phenotypic characteristics analyzed *in vivo* by Riedel where intact cells have been treated with EGF, it remains clear that these authors have not identified and appropriately characterized a responsive change in a phenotypic characteristic according to Applicant's teachings. As the Examiner has already pointed out, Riedel's results in some cases suggest that EGF is inhibitory for cell growth (p. 199, left hand column, third paragraph), whereas in other instances EGF was stimulatory for cell growth (p. 199, center column, first and second paragraphs, and Fig. 3). Under such conditions, cell growth *per se* is clearly not associated with overproduction of the POI in a predictable manner. As a result, the authors can formulate no conclusions regarding the ability of EGF, let alone any other test substance, to activate, inhibit, or have no effect whatsoever on the as yet undefined cellular functioning of the hybrid receptor in cells that express it.

For example, on page 199, left hand column, third paragraph, the authors determined that long term exposure of test cells expressing the hybrid receptor to EGF resulted in severe inhibition of growth as compared to control Rat 1 cells. Yet, like Rat 1 cells, the test cells express endogenous EGF receptors which stimulate growth in response to EGF. Using Riedel's disclosed test cells, the cellular functioning of the hybrid receptor cannot be distinguished from the cellular functioning of the endogenous receptor. The effect of EGF on the hybrid receptor expressing cells may be due to activation or inhibition of the hybrid receptor, or to inability of EGF to interact with and activate the endogenous EGF receptor (*e.g.*, EGF may simply be sequestered by the hybrid receptor and be unavailable to the endogenous EGF receptor). No conclusion may be drawn. The authors themselves, do not conclude that EGF is an activator or an inhibitor of the hybrid receptor (which they cannot do anyway). Rather, they conclude that it is a dose-dependent expression of the hybrid receptor itself that interferes with ("inhibits") the normal EGF effect on cells (*ibid*, last sentence of

paragraph). Since the authors have not demonstrated that the hybrid receptor is capable of exhibiting any responsiveness to EGF in terms of a cellular response (as distinct from mere autophosphorylation) they cannot attribute such effects to functional activation or inhibition of the hybrid receptor by EGF. Further, there is no explanation of how to overcome this core deficiency in order to arrive at a test cell that might be used according to Applicant's teachings.

Riedel refers to ligand responsiveness of their hybrid receptor expressing cells in terms of the ability of EGF to induce autophosphorylation. However, given the discussion above it remains to be seen whether EGF is activating or inhibiting (or having no direct effect on) the hybrid receptor at all. The authors concede this point, by stating on p. 199, left hand column, last paragraph, that "Further evidence that restoration of ligand-responsiveness did not convert v-*erb*B to a normal protein was the finding that transforming activity was not abolished in the chimera."

The authors go on to state in the next sentence that the remaining transforming activity, however, was now inducible by EGF. However their results indicate that the higher the level of expression of the EGFR-v*erb*B hybrid, the less transforming activity resulted. Thus, such a phenomenon is NOT the same as a graded cellular response, as the Examiner has suggested, because the alleged effect is reduced, not increased, with increasing expression of the hybrid receptor. Again, their results are entirely consistent with a mixed effect of EGF that includes activation of endogenous EGF receptors in Rat 1 cells to stimulate cell growth (a known effect), together with independent transformation by the cytoplasmic domain of v-*erb*B that is unaffected by EGF whatsoever.

Reidel's soft agar experiments further confirm that they have not identified a responsive change in a phenotypic characteristic according to Applicant's teachings. On p.199, bottom of left hand column; first and second paragraphs of middle column, in which the authors studied growth in soft agar exhibited by the hybrid receptor-expressing "test" cells, the "control" Rat 1 cells not expressing the hybrid EGF-v*erb*B construct and therefore not overproducing the hybrid protein were nevertheless also able to exhibit EGF-induced growth in soft agar. This is in stark contrast to the control cells in Applicant's method which

exhibit a clearly definable, measurable reduction in responsiveness of the selected phenotype response relative to Applicant's test cells. Applicant's control cells do not grow in soft agar and remain as single cells, even in the presence of a known PKC activator. (Specification, page 8, lines 14 - 15, Fig. 7, Panels A and B, and Table 2, page 53, line 16; '281 patent, Col. 4, lines 32-34 and Col. 20, line 37).

Thus, when all of these experimental results are taken together, it is apparent that Riedel did not define and appropriately characterize a reproducible *responsive change in a phenotypic characteristic* according to the teachings of Applicant's method. In Riedel's system, cell growth *per se* is not a phenotypic response according to Applicant's invention, as conflicting results are obtained depending upon whether the system is studied in cell culture or in soft agar. Reidel's sole stated interest was to attempt to define the functional domains of known receptors using a domain exchange approach. It was not their intention to develop a drug screening method in any event (A. Ullrich, personal communication with Applicant).

In addition to not having conceived or defined an appropriate responsive change in a phenotypic characteristic, there is no recognition of the second key concept of Applicant's method. Nowhere does Reidel disclose that once a responsive change in a phenotypic characteristic is appropriately identified, then the property may be utilized, most surprisingly and unexpectedly, as a tool to identify substances which interact with (bind to) the POI in order to inhibit or activate the cellular functioning of the POI. Indeed, since Riedel et al., have utilized only EGF in their experiments, which they know will bind to and activate the EGF receptor extracellular domain if the cell is capable of producing a functional version of said domain in the hybrid receptor, it would have been impossible for them to come to such a fundamental discovery and conclusion.

In summary, Riedel has neither conceived of nor identified a responsive change in a phenotypic characteristic, which is one of the two key elements of Applicant's invention. Further, even if one could be properly identified in retrospect using Riedel's hybrid receptor-expressing cell (Applicant respectfully asserts that the authors did not do so), there is no suggestion that such a responsive change can be used to identify substances that bind to and

inhibit or bind to and activate the hybrid receptor, which is the second key element of Applicant's invention.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 33-34, 36-37, 43-50, 59-65, 71-78 and 87-89 stand rejected under 35 U.S.C. § 112, first paragraph for lack of description in the specification. The Examiner has objected to two recitations that are present in each of the independent claims. First, the Examiner asserts that there is no support for a screening method wherein the POI is maintained such that the phenotypic response is exhibited by the cell after removal of the test agent. Second, the Examiner asserts that there is no support for use of a direct binding assay within the context of the disclosed invention.

The Level of the POI

Applicant previously amended the independent claims to recite cells that produce the POI and exhibit a phenotypic response to the POI, "wherein the level of the enzyme in the cell is maintained such that the cells capable of exhibiting phenotypic response following removal of a direct activator or direct inhibitor of the enzyme." The Examiner has asserted that the specification provides no support for generically practicing a screening method whereby expression of the POI is maintained in cells such that the phenotypic characteristic will be responsive to subsequent retesting.

Applicant respectfully traverses this rejection, emphasizing that the recited characteristic is a fundamental and inherent feature of the invention. As discussed above, the specification repeatedly discusses the importance of the level of expression of the POI and its relationship to the degree of the responsive change in a phenotypic characteristic that is observed in response to activators or inhibitors of the POI. The specification further illustrates the result (or lack thereof) when the level and activity of the POI is not controlled (*e.g.*, down regulation of PKC in response to treatment with a known activator; Specification, page 35, line 35 - page 36, line 1, '281 patent, Col. 16, lines 50-51; see also, Boreiko et al., 1980, Cancer Res. 40:4709-16). The end result of the down modulation or down regulation process is that levels of functional enzyme activity in the cell progressively disappear.

Following this loss of functional protein levels in the cell, the cell becomes refractory to additional attempts to stimulate PKC activity with a second treatment with the known activator. Unlike the situation with control cells however, Applicant's disclosed R6-PKC3 "test" cells are resistant to down modulation of PKC. Despite repeated treatments of the R6-PKC3 cells with the PKC activator TPA, *the cells continuously respond in the appropriate manner to each such treatment.* Specification, page 35, line 15 - page 36, line 5; '281 patent, Col. 16, lines 30-55.

Down-modulation is a well-known and common phenomenon that is seen with a wide variety of potential target proteins of interest for which potential therapeutic development may be directed. Thus, prior to Patentee's approach of overproduction of the POI to a sufficient extent to overcome the down-modulation problem, followed by definition and characterization of a responsive change in a phenotypic characteristic evoked by the overproduced protein in the cell under appropriate assay conditions, one simply could not create a generalized, reproducible POI-specific cellular assay system, since any resulting down-modulation of the POI following treatment with a substance would effectively preclude the ability to even define such a phenotypic characteristic and thus practice the method at all!

Patentee's method of overproducing the POI in the test cell such that the test cell produces higher, usually non-naturally occurring, levels of the POI ('281 patent, Abstract) overcomes the down modulation phenomenon in the general case. Maintenance of the POI such that the effects of down modulation are overcome is an inherent feature of the invention because in order to define and make use of a "responsive change in the phenotypic characteristic" (or "phenotypic response"), the phenomenon of down modulation should be sufficiently overcome such that the phenotypic response being utilized is definable and reproducible. As taught in the original specification and as the Applicant has emphasized repeatedly throughout prosecution of his issued patents, a "responsive change in a phenotypic characteristic" is, *by definition*, a phenotypic characteristic of the cell which is responsive to inhibitors or activators of the target protein. (See, e.g., Specification, page 23, lines 3 - 6; '281 patent, Col. 10, lines 26 - 29, Col. 18, lines 30 - 36; File wrapper, U.S. Patent 5,877,007, Declaration of Gerard M. Housey dated November 4, 1996, para. 10, second sentence

(Exhibit B); File wrapper, U.S. Patent 5,688,655, Amendment dated Jan. 18, 1995, page 6, last paragraph (Exhibit C)).

Thus, it is an inherent feature of the invention that the selection of a properly defined responsive change in phenotypic characteristic means the selection of an individual characteristic of the cell which is not adversely affected by the down modulation phenomenon. The initial selection of this individual characteristic of the cell (step 1) followed by the subsequent use (step 2) of this responsive property to identify chemical agents which interact with (bind to) the POI in order to inhibit or activate the POI's cellular functioning are the two core concepts of the Patentee's original invention.

In summary, it is a generic and inherent characteristic of Applicant's invention that the level of production of a POI is maintained in test cells after inhibition or activation of the POI by the test substance to such an extent that the test cell will be capable of exhibiting the phenotypic response after removal of the test agent during a subsequent retesting of the cell using the same starting conditions.[2] Accordingly, Applicant respectfully requests that the rejection be withdrawn.

A Direct Binding Assay is Superfluous

With regard to the necessity for a direct binding assay, Applicant notes that a method step reciting performance of a direct binding assay was added to the independent claims in response to the previous Office Action solely to address the Examiner's concerns about direct binding of a chemical agent to the POI. The Examiner had asserted that the preamble of the independent method claims required, *a priori*, knowledge concerning direct interaction, and further, that the Applicant had provided no means of determining whether an unknown agent would interact directly with the target enzyme and whether direct interaction was responsible for an observed phenotypic change. Applicant amended the preamble to remove any ambiguity as to *a priori* knowledge. More significantly, to facilitate allowance, Applicant added the binding assay limitation, but maintained that such a binding assay was optional and not required for generic embodiments of the invention.

[2] One of skill in the art would understand that in order to retest such cells, it might be necessary for the phenotypic response to first return to its basal level.

The independent claims are currently amended so as not to recite a binding assay step. As discussed above, the method is inherently capable of identifying substances that are direct activators or inhibitors of a given POI such as an enzyme in a cell. That is, in distinct contrast to prior screening methods, chemical agents that are found to modulate the phenotypic characteristic capable of being evoked by the POI in the test cell do so by binding to the POI and modulating the activity of the POI. The method does not require the concomitant use of any separate cell free or intact cell binding assay to accomplish its claimed purpose of determining whether a chemical agent is a direct inhibitor or activator of an enzyme in a cell. As previously pointed out, the essence of the invention is the capability to identify compounds that bind to (interact with) a target POI and inhibit or activate the cellular functioning of the POI. This is demonstrated in the instant specification by the conclusive and irrefutable identification, for the first time, of the chemical agent tamoxifen as a substance which inhibits the ß1 isoform of protein kinase C by interacting with (binding to) this isoform of this enzyme to inhibit its cellular functioning (Specification, page 39, lines 21-26; '281 patent, Col. 18, lines 30-36). As discussed during the meeting with the Examiner on May 12, 2003 and elsewhere, tamoxifen's ability to bind to and inhibit PKC was independently verified using alternative techniques in an after-published manuscript. (O'Brian, Housey et al., 1988, Cancer Res. 48:3626-29; page 3626, paragraph spanning left and right columns; previously provided).

Applicant respectfully points out that subsequent to Applicant's original disclosure, other investigators have shown that functional cell-based assay systems using a cellular response-based readout according to Patentee's method (rather than mere radioligand binding) are capable of identifying compounds which hit (bind to) the POI in order to inhibit or activate its cellular functioning. Moreover, as Applicant first proved, such functional cell-based assays are often superior to cell-free binding studies. For completeness, Applicant provides a representative sample of such references. In several cases, the cell-based assays are compared to traditional radioligand binding assays.

1) U.S. Patent 6,107,297 (Exhibit D), originally field Jun. 25, 1998 as PCT/SE98/01240, entitled "2,4 Dithi(oxo)-pyrimidin-5-yl Compounds Bearing a Tricyclic

Substituent Useful as P2 Purinoceptor Antagonists," describes a functional cell-based assay system according to the teachings of Applicant, used to:

> determine how strongly [emphasis added] the compounds of the invention bind to P2-purinoceptor 7-TM G-protein coupled receptors. The assay used a human P2Y2 receptor clone which was isolated from HL60 cells cDNA and then stably transfected into a Jurkat cell line (using methods described in "Cloning and Characterisation of a Bovine P2Y Receptor" Henderson et al., (1995), 212, 2, 648-656; Parr et al., Proc. Natl. Acad. Sci. USA (1994) 91, 3275-3279 and Proc. Natl Acad Sci USA (1994), 91, 13067). The cloned receptor mediates an increase in intracellular calcium in the cell line, which possesses no endogenous nucleotide receptor of its own.

U.S. Patent 6,107,297, Col. 45, line 66 - Col. 46, line 11.

2) Daniels et al., Eur. J. Pharmacol. 370 (1999) 337-343 (Exhibit E), utilize a functional cell-based assay system expressing various human cloned α_{1A}-adrenoceptor isoforms (α_{1A-1}, α_{1A-2} and α_{1A-3} isoforms) to estimate antagonist affinities, a quantitative assessment of the strength of a compound's ability to bind to a target POI. The authors determine the agonist affinities of various compounds using CHO cells expressing each of the various adrenoceptor isoforms by assaying the levels of inositol phosphate production following noradrenaline stimulation. Note that on page 340, in columns 1-4 of Table 2, entitled "Agonist affinity estimates at α_{1A}-adrenoceptor isoforms from functional studies (noradrenaline-stimulated InsPs accumulation)," affinity estimates as determined by the functional cell-based assay system are compared to traditional radioligand binding assays (given in the sixth column of the table).

3) Kehne et. al., J. Pharm. Exp. Ther., 277 (1996) 968-981 (Exhibit F), use fibroblast cell lines stably expressing the rat 5-HT_{2A} and 5-HT_{2C} receptors. The authors assay inositol phosphate accumulation to determine agonist/antagonist activity and receptor selectivity, both of which are properties that necessitate binding of the substance to the POI. On page 972, right hand column, last paragraph, the authors state that

> 5-HT induced [^{3}H] inositol phosphate accumulation in fibroblast cell lines transfected with the rat 5-HT_{2A} and 5-HT_{2C}

> receptors was used to determine agonist/antagonist activity and receptor selectivity in vitro.

4) In U.S. Patent 6,229,000 (Exhibit G), a continuation-in-part of an application originally filed May 19, 1993), entitled "Tissue-Specific Human Neuronal Calcium Channel Subtypes and Their Use" the authors create recombinant cell systems expressing human neuronal calcium channel subtypes and utilize calcium flux assays to identify potential calcium channel antagonists or agonists. The inventors state in column 3, lines 1-3 that "[t]his recombinant cell system can be used as a functional test for finding sub-type-specific calcium channel ligands (agonists and antagonists)." At Col. 3, lines 5-12, the authors state:

> The calcium fluxes produced by membrane depolarisation can be measured electrophysiologically (example: Carbone et al., 1990, Pflugers Arch., 416: 170-179). With the aid of the recombinant animal cell lines (see above), human calcium channels can be used for direct physical measurement and pharmacological characterisation of the effect of potential calcium channel antagonists or agonists.

5) In Grimwood et. al., J. Neurochem., 66 (1996) 2589-2595 (Exhibit H), the authors create cell lines stably expressing recombinant human NMDA receptors and measure modulations of Ca++ influx in response to treatment with various compounds. The authors state on page 2593, right hand column, "Discussion" section, second sentence:

> This assay is advantageous because it allows the investigation of interactions at any of the modulatory sites on the NMDA receptor [emphasis added] and avoids problems associated with homomeric subunit assemblies encountered with radioligand binding studies.

The authors conclude on page 2595, left hand column, first full paragraph that they have:

> . . . developed a functional assay that measures the degree of $^{45}Ca^{++}$ influx into cells stably expressing NR1a/NR2A and NR1a/NR2B receptors. This technique can be used to investigate, or detect, the interaction of NMDA receptor ligands at any of the receptor's modulatory sites [emphasis added].

6) In Kargman et. al., Biochem. Pharm., 52 (1996) 1113-1125 (Exhibit I), the authors create CHO cells expressing human prostaglandin G/H synthase-1 and –2 and assay

for the ability of various compounds to inhibit prostaglandin production in these cells. On page 1123, right column, last paragraph, the authors state:

> Purified ram seminal vesicle PGHS-1 and recombinant cell-free preparations of PGHS isoforms have been essential in establishing mechanisms of inhibition and initial profiles of PGHS inhibitors. However, several groups have identified the requirement to test inhibitor potency in whole cells as cell-free systems appear to often underestimate the potency and selectivity of PGHS inhibitors.

Potency refers to the extent to which a given substance is capable of activating or inhibiting a given POI. Selectivity refers to the ability of an activator or inhibitor to modulate one isoform of a closely related protein family member preferentially over other member(s) in the protein family. Thus, the authors point out the benefit of employing a cell-based assay to determine selectivity and potency of substances that interact with (bind to) their POI.

7) Hoffmann La Roche (Roche), now one of Applicant's largest licensees, previously brought a declaratory suit in Switzerland against Applicant's issued European counterpart patent, which is essentially identical to the '281 patent. Roche's own description of the Applicant's invention is illuminating, to say the least:

> The broad degree of identity between the test cell line and the control cell line ensures that the effect of the substances to be investigated for their inhibitor or activator characteristics can be carried out specifically for the POI, and that the results of the tests cannot be falsified by alterations to the test cell line which cannot be attributed to the effect of the substances being investigated on the POI. The high selectivity of the method in accordance with the patent sued upon is (together with the simplicity of execution) the main advance provided by the invention as compared with the state of the art.

Exhibit J, para. 41.

In summary, it is now clear that Applicant's invention is capable of accomplishing what was previously thought to be impossible. Moreover, the significance of Applicant's cell-based assay method is reflected by the fact that over 70% of the world's top 50 research based pharmaceutical companies have licensed the 4 U.S. and 24 world-wide patents and

pending applications covering cell-based assay technology from Housey Pharmaceuticals, Inc.

Rejection Under 35 U.S.C. § 112, second paragraph

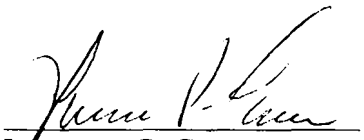
Claims 87-89 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite for use of an improper form for a multiple dependent claim. Applicant has reformatted the claims in view of the Examiner's recommendation. The rejection is believed moot and Applicant respectfully requests that the rejection be withdrawn.

Conclusion

It is believed that this amendment is fully responsive to the Examiner's rejections. In view of the foregoing amendments and remarks, it is firmly believed that the subject claims are in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

KENYON & KENYON

Date: October 22, 2003 By: 

Lawrence P. Casson
Reg. No. 46,606

One Broadway
New York, NY 10004
Telephone: (212) 425-7200
Facsimile: (212) 425-5288